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=> s Alkaline lipase and Vibrio metschnikovii  
36 FILES SEARCHED...

L1 7 ALKALINE LIPASE AND VIBRIO METSCHNIKOVII

=> dup rem 11

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L2 ANSWER 1 OF 5 IFIPAT COPYRIGHT 2004 IFI on STN DUPLICATE 1  
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TITLE: **ALKALINE LIPASE FROM**  
**VIBRIO METSCHNIKOVII RH530 N-4-8**

INVENTOR(S): AND NUCLEOTIDE SEQUENCE ENCODING THE SAME  
Jhon; Sung Hoo, Seoul, KR

Jin; Ghee Hong, Seoul, KR

Lee; Hyun Hwan, Yongin-City, KR

Rho; Hyune Mo, Seoul, KR

PATENT ASSIGNEE(S): Unassigned

AGENT: Cantor Colburn LLP, 55 Griffin South Road,  
Bloomfield, CT, 06002, US

NUMBER	PK	DATE
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PATENT INFORMATION: US 2004009570 A1 20040115

APPLICATION INFORMATION: US 2003-603260 20030624

NUMBER	DATE
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PRIORITY APPLN. INFO.: KR 2002-35410 20020624

FAMILY INFORMATION: US 2004009570 20040115

DOCUMENT TYPE: Utility

Patent Application - First Publication

FILE SEGMENT: CHEMICAL

APPLICATION

NUMBER OF CLAIMS: 12 10 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 shows a recombinant vector pH1 containing 3.2 kb DNA insert (vail) having an **alkaline lipase** gene according to the present invention;

FIG. 2 shows an agarose gel electrophoresis of the recombinant vector pH1 having an **alkaline lipase** gene according to the present

invention, in which M denotes a size marker, lane 1 has a supercoiled type pUC19, lane 2 has a pUC19 digested with HindIII, lane 3 has a recombinant vector pHL1 digested with HindIII, the band of 2.7 kb corresponding to a vector pUC19 and the band of 3.2 kb corresponding to a DNA insert containing the \*\*\*alkaline\*\*\* **lipase** gene according to the present invention, and lane 4 has a supercoiled type recombinant vector pHL1; FIG. 3A shows an agarose gel electrophoresis of a DNA fragment containing the \*\*\*alkaline\*\*\* **lipase** gene according to the present invention, and FIG. 3B shows a photograph of Southern blotting, in which M denotes a size marker marked by DIG, lane 1 has **Vibrio metschnikovii** chromosomal DNA, lane 2 has **Vibrio metschnikovii** chromosomal DNA digested with HindIII, lane 3 has **Vibrio** \*\*\*metschnikovii\*\*\* chromosomal DNA digested with AvaI and EcoRI, lane 4 has pUC19 digested with HindIII, lane 5 has a supercoiled type recombinant vector pHL1, lane 6 has a recombinant vector pHL1 digested with HindIII, and lane 7 has recombinant vector pHL1/AvaI and EcoRI (probe); FIGS. 4A and 4B show a base sequence of a DNA insert containing the \*\*\*alkaline\*\*\* **lipase** gene from **Vibrio** \*\*\*metschnikovii\*\*\* RH530 N-4-8 according to the present invention, a regulatory element and an amino acid sequence derived therefrom; FIG. 5 shows a restriction enzyme map from which a minimum length and a gene position for expression of the **alkaline lipase** according to the present invention are identified in the DNA insert of the recombinant vector pHL1; FIG. 6 shows the comparison result of an amino acid sequence deduced from the \*\*\*alkaline\*\*\* **lipase** gene according to the present invention with *Pseudomonas glumae*, and *Burkholderia cepacia*; FIG. 7A shows a restriction enzyme map of a region prior to the promoter of the \*\*\*alkaline\*\*\* **lipase** gene according to the present invention, and FIG. 7B shows a change in activity when the region prior to the promoter is removed using the restriction enzyme; FIG. 8A shows a change in activity of the **alkaline lipase** according to the present invention, and FIG. 8B shows the measuring result of residual activity of the **alkaline lipase** according to the present invention depending on temperature; FIG. 9A shows a change in activity of the **alkaline lipase** according to the present invention depending on pH, and FIG. 9B shows the measuring result of residual activity of the **alkaline lipase** according to the present invention depending on pH; and FIG. 10 shows the effect of surfactant or detergent on the activity and stability of the **alkaline lipase** according to the present invention, for which enzyme solutions mixed with sodiumolefinsulfonate (AOS) (FIG. 10A), sodium alkylbenzen-sulfonate (LAS) (FIG. 10B) and sodium dodecyl sulfate (SDS) (FIG. 10C) are spotted on a 0.5% tricaprylin medium.

AB An **alkaline lipase** isolated from **Vibrio metschnikovii** RH530 and a polynucleotide sequence encoding the same are provided. The isolated **alkaline lipase** has an amino acid sequence of SEQ ID NO: 5 and the polynucleotide having a base sequence of SEQ ID NO: 4 encodes the **alkaline lipase**. The isolated **alkaline lipase** exhibits an optimal activity at a high pH level, that is, at pH 10\*11, and has very high ratio of residual enzyme activity and high compatibility with a surfactant, so that it can be suitably used as an enzyme for a laundry detergent.

L2 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2004-05981 BIOTECHDS

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** RH530 N-4-8;  
recombinant enzyme production in *Escherichia coli*

AUTHOR: JIN G; JHON S; LEE H; RHO H  
PATENT ASSIGNEE: CJ CORP  
PATENT INFO: WO 2004001029 31 Dec 2003  
APPLICATION INFO: WO 2003-KR1227 23 Jun 2003

PRIORITY INFO: KR 2002-35410 24 Jun 2002; KR 2002-35410 24 Jun 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-082499 [08]

AB DERWENT ABSTRACT:

NOVELTY - An alkaline lipase (I) isolated from **Vibrio metschnikovii** RH530 N-4-8 comprising a fully defined sequence of 185 amino acids (S1) as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) polynucleotide (II) encoding (S1); (2) recombinant vector (III) comprising (II); (3) host cell (IV) transformed by (III); and (4) detergent comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) (claimed). Preferred Polynucleotide: (II) comprises a fully defined sequence of 555, 798 or 2578 base pairs as given in the specification. Preferred Recombinant Vector: (III) is pHL1, pHLB29 or pHAAH38.

USE - (I) is useful as an enzyme for laundry detergent (claimed).

ADVANTAGE - (I) has high residual enzyme activity and high compatibility.

EXAMPLE - Culture medium comprising tryptone, yeast extract, sodium chloride in sodium carbonate buffer was used for culturing **Vibrio metschnikovii** RH530 N-4-8, at 30 degreesC. The cells were collected and treated with lysozyme to lyse the cell. The resultant product was treated with phenol and chloroform to remove protein, and a precipitate was removed by centrifugation. A **Vibrio** chromosomal DNA was obtained from the supernatant. The obtained chromosomal DNA was cut with a restriction enzyme HindIII to be recombined with a cloning vector pUC19, followed by transforming **Escherichia coli** HB101, thus cloning a DNA fragment containing a 3.2 kb **alkaline lipase** gene. The resulting recombinant vector was referred to as a vector pHL1. After treatment with the restriction enzyme HindIII, an electrophoresis with 1% agarose gel was performed. The agarose gel electrophoresis showed that the **alkaline lipase** gene was cloned. To confirm that a DNA fragment containing an **alkaline lipase** gene derived from **V. metschnikovii**, which is contained in a recombinant vector pHL1, is identical with the gene from **V. metschnikovii**, Southern blotting was performed. DNA of 3.2 kb was treated with an exonuclease Bal31 to subclone the same in a minimum length required for expression of a lipase. Production of the lipase was confirmed by formation of a clear halo, and the result of subcloning showed that 2.6 kb DNA fragment was necessary for lipase activity. The recombinant vector containing such a gene having a minimum length was referred to as pHLB29. DNA of 2.6 kb fragment was subcloned in a direction opposite to that of a SmaI site of pUC19, and referred to as pHAAH38. Although the 2.6 kb DNA fragment was subcloned in a reverse direction relative to a lac promoter, pHAAH38 produced a clear halo at a tricaprylin culture medium, confirming that an **alkaline lipase** promoter existed in the 2.6 kb DNA fragment and the promoter used when it is transcribed from **E. coli**. (35 pages)

L2 ANSWER 3 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: ADH51272 protein DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** RH530 N-4-8.

INVENTOR: Jin G; Jhon S; Lee H; Rho H

PATENT ASSIGNEE: (CJCJ-N) CJ CORP.

PATENT INFO: WO 2004001029 A1 20031231

35p

APPLICATION INFO: WO 2003-KR1227 20030623

PRIORITY INFO: KR 2002-35410 20020624

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-082499 [08]

CROSS REFERENCES: N-PSDB: ADH51271

DESCRIPTION: **Vibrio metschnikovii** lipase chaperone protein.

AB The present sequence is the protein sequence of a putative chaperone protein, denoted **Vall1**, for an **alkaline lipase** **ADH51273**, denoted **Vall2**, of **Vibrio metschnikovii** **RH530 N-4-8**. It is encoded by an open reading frame that is controlled by the same promoter sequence as the lipase open reading frame. Homology comparisons suggested that **Vall2** is a lipase while **Vall1** is a lipase chaperone or is the product of an auxiliary gene for extracellular secretion. Lipase **Vall2** can be obtained by recombinant production, especially in transformed *Escherichia coli* **HB101** (**pHL1**) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, showing it to be potentially useful as an additive for a laundry detergent.

L2 ANSWER 4 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: **ADH51273** protein DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** **RH530 N-4-8**.

INVENTOR: **Jin G; Jhon S; Lee H; Rho H**

PATENT ASSIGNEE: **(CJJCJ-N) CJ CORP.**

PATENT INFO: **WO 2004001029 A1 20031231**

35p

APPLICATION INFO: **WO 2003-KR1227 20030623**

PRIORITY INFO: **KR 2002-35410 20020624**

DOCUMENT TYPE: **Patent**

LANGUAGE: **English**

OTHER SOURCE: **2004-082499 [08]**

CROSS REFERENCES: **N-PSDB: ADH51271**

DESCRIPTION: **Vibrio metschnikovii** **alkaline** **lipase**.

AB The present sequence is the protein sequence of an **alkaline lipase**, denoted **Vall2**, from **Vibrio metschnikovii** **RH530 N-4-8**. The lipase can be obtained by recombinant production, especially in transformed *Escherichia coli* **HB101** (**pHL1**) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, making it potentially useful as an additive for a laundry detergent.

L2 ANSWER 5 OF 5 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN

ACCESSION NUMBER: **ADH51271** DNA DGENE

TITLE: **Alkaline lipase** useful as laundry detergent, isolated from **Vibrio metschnikovii** **RH530 N-4-8**.

INVENTOR: **Jin G; Jhon S; Lee H; Rho H**

PATENT ASSIGNEE: **(CJJCJ-N) CJ CORP.**

PATENT INFO: **WO 2004001029 A1 20031231**

35p

APPLICATION INFO: **WO 2003-KR1227 20030623**

PRIORITY INFO: **KR 2002-35410 20020624**

DOCUMENT TYPE: **Patent**

LANGUAGE: **English**

OTHER SOURCE: **2004-082499 [08]**

CROSS REFERENCES: **P-PSDB: ADH51272; ADH51273**

DESCRIPTION: **Vibrio metschnikovii** **alkaline** **lipase** **gene**.

AB The present sequence comprises a fragment of **Vibrio metschnikovii** **RH530 N-4-8** chromosomal DNA found in vector **pHL1**. This vector was obtained by **HindIII** digestion of **RH530 N-4-8** DNA and insertion of digested fragments into cloning vector **pUC19**. The sequence comprises 2 open reading frames, **vall1** and **vall2** (also claimed), existing under a single promoter. Homology comparisons suggested that **vall2** encodes a lipase while **vall1** encodes a lipase chaperone or is an

auxiliary gene for extracellular secretion. The lipase can be obtained by recombinant production, especially in transformed *Escherichia coli* HB101 (pHL1) host cells. It exhibits maximum activity at 50-60 degrees C and pH 10-11, and has resistance against 0.07% sodium alkylbenzene-sulfonate, 0.1% sodium-alpha olefin sulfonate and 0.1% sodium dodecyl sulfate, showing it to be potentially useful as an additive for a laundry detergent.

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